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Carcinogenic Potential of Rotenone. Phase I: Dietary Administration to Hamsters

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**Phase I:
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Administration to
Hamsters**

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CARCINOGENIC POTENTIAL OF ROTENONE
PHASE I: DIETARY ADMINISTRATION TO HAMSTERS

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

Rotenone, the extract of derris root, has long been used as an insecticide and fish poison. A recent report by the Spanish investigators Gosalvez and Merchan has shown rotenone to produce mammary tumors in rats by intraperitoneal or dietary dosage. The present study was carried out to determine the possible carcinogenic effect of rotenone in hamsters.

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ABSTRACT

Studies were performed to evaluate the potential carcinogenicity rotenone in the Syrian Golden hamster. Several ancillary range-finding studies were carried out including 14-day feeding trials and a reproduction experiment. The latter experiment indicated that rotenone at a level of 500 ppm in the maternal diet was embryo-toxic (depressed birth weight and fetal deaths) and resulted in cannibalism of the young by the maternal animals. A level of 1000 ppm led to sterility in one or both sexes. The 18-month carcinogenesis study indicated that 1000 ppm rotenone in the diet was toxic to hamsters (depressed body weights compared to controls) but no gross or histopathological evidence was obtained which indicated that the test compound induced the formation of any tumors. Three adrenal cortical carcinomas in 65 hamsters observed in the 1000 ppm group could not be ascribed to rotenone treatment.

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INTRODUCTION

In a brief communication published in 1973 (Cancer Research 33:3047), Gosalvez and Merchan described experiments in which mammary tumors were found in rats which were fed rotenone. In these studies, 4 series of female albino rats of a strain which reportedly has a natural mammary tumor incidence of 0.5 tumors per 1000 rats per year, were given intraperitoneal injections of rotenone (K & K Laboratories, Inc., Plainview, N.Y., 1.7 mg/kg body weight) dissolved in 0.1 ml sunflower oil on 42 consecutive days. Control animals received 0.1 ml of vehicle. At study initiation the rats were 35 days old (approximately 100 grams each), and were observed for up to 19 months after treatment. In the first series, 8 out of 8 rats exhibited mammary tumors between months 6 and 11 after treatment while 10 controls had no tumors after 10 months. Several of these tumors were histologically diagnosed as "adenomas with accentuated interstitial fibrosis and showed localized areas with adenocarcinomatous transformation: 1 was diagnosed as a differentiated adenocarcinoma. All tumors were encapsulated and did not show metastasis." Four to five successful tumor transplants out of 30 trials were made to normal rats. Primary and transplanted tumors developed slowly, requiring 7 to 12 months after initial detection before full development was achieved.

This report presents results of a chronic feeding study which was performed to investigate the carcinogenic potential of rotenone in Syrian Golden hamsters. Included are observations made in ancillary studies on effects of rotenone on hamster reproduction and acute toxicity trials.

The following page presents an outline of the different phases of the study, and the dates associated with each phase.

OUTLINE OF HAMSTER STUDIES

<u>Title & Description</u>	<u>Dates</u>		
	<u>Receipt of Test Animals</u>	<u>Initiation</u>	<u>Completion</u>
<u>Prechronic Study I</u>	8/25/75	9/3/75	10/9/75
To test feed palatability and subacute toxicity of rotenone: rotenone administered 14-days in feed at 63, 125, 250, 500 and 1000 ppm. After 14-day observation period, gross necropsies performed.			
<u>Prechronic Study II</u>	9/23/75	10/7/75	11/11/75
Repeat of prechronic Study I. This study included addition of corn oil to rotenone-feed mixtures to increase chemical stability.			
<u>Oral Dosing Study</u>	9/16/75	9/30/75	10/8/75
Rotenone was administered by oral gavage in corn oil for 9 days in a dose of 80 mg/kg. Animals observed for gross toxic signs			
<u>Reproduction Study</u>			
Both males and females maintained on rotenone diets: litter sizes, offspring survival and pregnancy rates were recorded.			
Group Fed 1000 ppm	12/3/75	12/8/75	6/1/76
Group Fed 0 ppm	3/16/76	3/21/76	1/26/77
Group Fed 500 ppm	6/15/76	6/28/76	1/25/77

OUTLINE OF HAMSTER STUDIES
(Continued)

Title & Description

<u>Receipt of Test Animals</u>	<u>Dates</u>	
	<u>Initiation</u>	<u>Completion</u>

Carcinogenesis Study

Hamsters fed rotenone in
diets for 18 months;
histopathology performed on
0, 125, and 1000 ppm groups.

Group Fed 0 ppm	1/13/76	1/26/76	7/28/77
Group Fed 125 ppm	1/27/76	2/9/76	8/17/77
Group Fed 250 ppm	1/21/76	2/2/76	7/29/77
Group Fed 500 ppm	1/6/76	1/19/76	7/22/77
Group Fed 1000 ppm	12/29/75	1/12/76	7/15/77

OBJECTIVES, METHODS, AND OBSERVATIONS

Rotenone Purity and Dose Preparation

Rotenone employed in this study was obtained from S. S. Penick and Co., Orange, New Jersey. Analysis of the chemical by high pressure liquid chromatography at Battelle according to published procedures [J. Chromatography 134:207 (1976)] determined the rotenone to be 95+ percent pure. The stock chemical was stored at -20°C under nitrogen prior to dosage preparation.

Rotenone was prepared for oral gavage by adding appropriate amounts of the chemical to corn oil, followed by high-speed stirring of the mixture. Corn oil (Mazola) was obtained locally from retail grocery stores, and was used without purity analyses.

Dosed feed preparations for Prechronic Study I were prepared by adding rotenone to Purina Laboratory Chow Meal, and mixing for 30 minutes in a Patterson-Kelly twin shell blender. For subsequent studies, rotenone was first suspended in corn oil and then blended with the feed. By instituting the addition of a one percent corn oil concentration in the feed mixtures, the stability and homogeneity of the test chemical were increased. Ninety-two percent of added rotenone could be recovered after one week by employing this procedure. Feed mixtures for the Prechronic II, Reproduction and Carcinogenesis studies contained one percent corn oil, and were prepared weekly throughout the studies.

Coded feed samples were sent to Penick Research and Applications Development Center (Mr. Joseph Haus) for rotenone analyses. Values obtained from these assays were generally within 20 percent of calculated values except at the higher concentrations in which cases decreased extraction efficiency was suspected as the cause of the low values. See Appendix I for these results.

Hamster Care and Handling

Animals were housed individually throughout all studies except during mating periods in the reproduction study. Hamsters were housed in 19 X 10.5 X 6.2 inch polycarbonate cages in rooms separate from those

used for other studies. Cage bedding was changed once per week. Environmental conditions were maintained under conditions suitable for the species as outlined in Battelle's Manual on Standard Operating Procedures for Animal Housing and Care, including temperature, 70°-74° and relative humidity, 50-60%.

The Syrian Golden hamsters used in these studies were obtained from Charles River Lakeview Hamster Colony. Each group of animals was given a minimum of five days for quarantine before going on study. Water was provided ad libitum throughout. Animal cages were equipped with feeders that were suitable for periodic feed consumption determinations. Hamsters were uniquely identified by a toe clipping-ear punching scheme.

Data and Test Material Storage

All records, tissues, paraffin blocks, microscopic slides, and reports for this study will be stored in Battelle's archives.

Prechronic Study I

This four-week study was carried out prior to beginning chronic studies to provide preliminary information on the palatability of rotenone-containing feeds, and any resulting gross toxicity which may occur in the treated animals.

Methods

Rotenone was blended with Purina Hamster Chow Meal to yield concentrations of 63, 125, 250, 500, and 1000 ppm. Hamster weights ranged from 50 to 70 grams at the beginning of the study. Animals were given access to control feed for 1 week prior to dosing. Hamsters in groups of 10 animals, (5 males and 5 females) were then fed rotenone-containing feeds for 14 days, followed by 14 days of control feed. Animal body weights and feed consumption were determined weekly. Gross necropsies were performed 14 days following final exposure to rotenone.

Observations

The mean body weight data indicate that there was a substantial reduction in weight gain for males during the first week of feeding 1000 ppm rotenone in the diet (Figure 1.) The effect was reversible with observed weight gains greater than normal after return to control diet.

During the course of the preliminary study, no abnormal behavior or symptomology was observed.

Gross observations at necropsy showed lungs of several 1000 ppm hamsters to be congested and colored cherry red. Several small petechial hemorrhagic areas were present on the lung surface. Also seen were congestion of the duodenal mucosa, dilation of meningeal vessels and hemorrhagic enteritis in the small intestines. Similar but less extensive lesions were present in animals given 500 ppm. No lesions were found in the 250 ppm or lower dosage groups. None of the hamsters died during the course of the study.

Prechronic Study II

A second range-finding feeding study was performed in an identical manner to Study I except corn oil was used as a vehicle for dispersing rotenone into the feed. This procedure was designed to provide for more homogeneity and stability of the chemical in the feed.

Methods

Rotenone was suspended in corn oil and blended with Purina Lab Chow. Final rotenone concentrations were 0, 63, 125, 250, 500, and 1000 ppm, and corn oil was one percent. Ten hamsters were assigned to each feeding group, 5 males and 5 females. Animals were fed control diet for 1 week, rotenone diets 2 weeks, and again control diets for 2 weeks. Weekly body weights and feed consumptions were recorded. Necropsies were performed on five 1000 ppm and two 500 ppm hamsters.

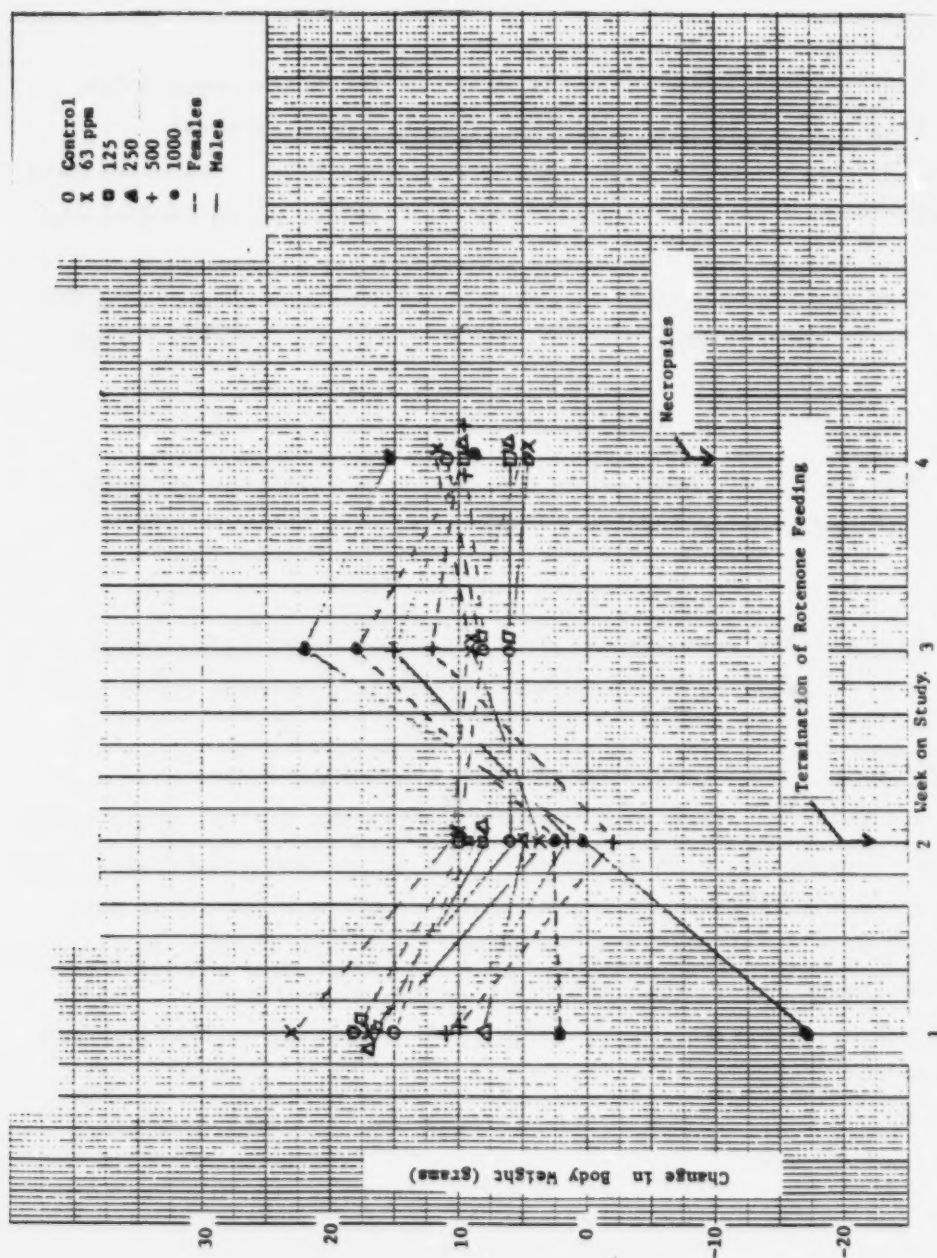


FIGURE 1. Hamster Group Mean Body Weights During Prechronic Study I

Observations

Figure 2 shows that no substantial differences occurred for mean body weight gains between treatment groups. No behavioral changes or deaths were evident during the four week study. No detectable lesions were found during gross necropsies carried out at the end of the study.

This preliminary study confirmed that 1000 ppm rotenone in the diet would be an appropriate high dose level to use in a chronic study.

Oral Dosing Study

This study was designed to determine the toxicity following oral gavage of rotenone to hamsters.

Methods

Ten hamsters were given oral intubations of 80 mg/kg rotenone in 1.0 ml of corn oil daily. Observations and dosing continued for 9 days.

Observations

The animals soon became lethargic after the initial dose. Three animals died during the first two days of the study, and exhibited an oily substance in the peritoneal cavity, although no stomach punctures were found. Other animals displayed signs of diarrhea, eye discharge, lameness, shaggy coats, and mouth ulcerations. The lungs, liver, and G.I. tract were congested with redness in the lungs and pleura. Intussusceptions of the small intestine and rectal prolapses were commonly seen. Rats treated with corn oil only exhibited mild diarrhea for four days which subsided after that time.

Reproduction Study

In an effort to evaluate the toxic effects of rotenone on reproduction, the following study was initiated. Male and female animals were placed on rotenone diets, and the effects on number of offspring,

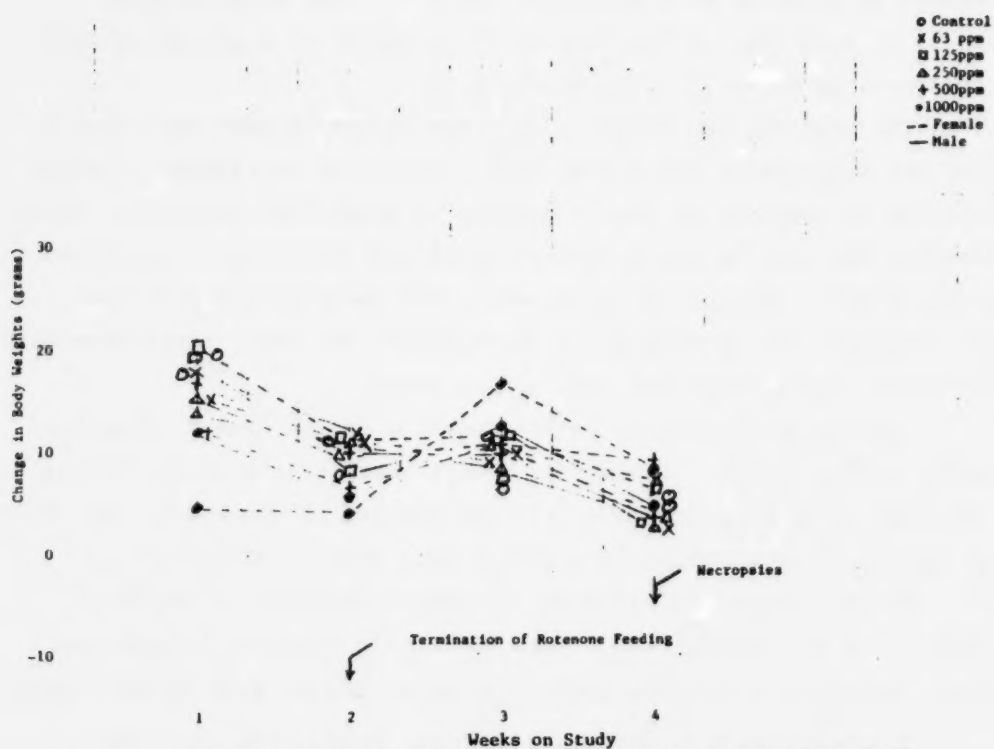


FIGURE 2. Group Mean Hamster Body Weights During Prechronic Study II

number of pregnancies, and sex ratios for offspring were to be evaluated. F₁a offspring were to be maintained on rotenone diets to determine their sensitivity to any potential carcinogenic effects of the chemical.

The 1000 ppm Diet Study

The first group of animals (mean body weights were 45 grams for both sexes) to be mated were maintained on a 1000 ppm rotenone diet containing 1% corn oil. Fifty females and 25 males were placed on test diet, and were to be mated three months later.

The hamsters continued to increase in body weight the first 5 weeks of the study after which time feed consumption decreased. Sixteen (3 males and 12 females) of the 75 animals on study died during the first two months. Physical condition deteriorated and body weights decreased in the survivors. At eight to nine weeks, the downward trend in body weights reversed, and animals became more alert, and their coats developed a sheen while feed consumption returned to normal.

Females were observed daily according to the Orsini technique to monitor estrus cycles. During the fourth month of the study, mating was initiated by housing one male with two females at the end of the first day of the estrus cycle which is normally four days in duration.

Several vaginal plugs were observed. However, it was soon determined that one or both sexes were nonfertile since no pregnancies occurred. Males were observed grossly to have smaller than normal testicles.

Since attempts to produce offspring in the 1000 ppm treated hamsters were unsuccessful, the study was terminated, and a lower dietary level was scheduled for a subsequent study.

The 0 ppm Diet Study (Controls)

Hamsters (50 males and 50 females) were maintained on a control diet (1% corn oil in Lab Chow Meal) throughout the entire study. One female was caged with one male for mating. Pregnancies occurred, and offspring were delivered.

The F_{1a} generation all appeared healthy through weaning. Forty-three litters were delivered from 50 females. Average litter size was 7 with a range from 2 to 10 pups. One to three pups were retained from each litter, and placed in the test group to be observed for tumor formation.

The second group of offspring (F_{1b}) also appeared to be healthy through weaning. There was an average of 12 pups per litter which ranged in size from 9 to 17 pups.

The 500 ppm Diet Group

Fifty males and fifty females were maintained on a 500 ppm rotenone diet containing 1% corn oil. The animals were fed test diet for three months prior to and during the mating periods. Mating was carried out as described previously.

The first generation of offspring (F_{1a}) consisted of 45 litters from 50 females. Only 7 survived through weaning as the dam often cannibalized or totally neglected her young. The pups were all smaller than normal. Average litter size was 9 pups, and ranged from 4 to 15 offspring.

The second litters from the 500 ppm group consisted of 21 litters, ranging in size from 7 to 16 pups with an average of 12. The dams continued to refuse to nurse their young and often cannibalized them.

Six months after the beginning of the 500 ppm rotenone feeding study, and 10 months for the 0 ppm group, the studies were terminated concurrently at the request of the EPA Project Officer. The high toxicity of rotenone at the 500 ppm level as exhibited by large numbers of dead births, maternal deaths, cannibalism of offspring, and offspring deaths resulted in a small number of hamsters surviving weaning, and continuation of the study at this dose level would not be productive.

Carcinogenesis Study

A long term (18-month) study to evaluate the potential carcinogenicity of rotenone in hamsters was performed. Rotenone was incorporated in the feed of the test animals for the duration of the study.

Methods

Hamsters were divided into five groups of 50 males and 50 females each. The groups were fed diets containing 1% corn oil and rotenone concentrations of 0, 125, 250, 500, or 1000 ppm. Animals were weighed weekly for the first 6 months, and bi- or tri-weekly thereafter. Feed consumptions were measured weekly throughout the study. Daily observations were made for mortality, behavior and appearance.

All hamsters in the feeding study (with the exception of animals numbered 223 and 283 from the 0 ppm group, number 118 in 500 ppm group, and numbers 63 and 82 from the 1000 ppm group) were subjected to complete necropsy and examined for evidence of tumors at the time of their spontaneous death, or following sacrifice with intraperitoneal injection of pentobarbital sodium at the termination of the study. The above five animals were inadvertently lost to necropsy evaluation early in the study.

Tissues examined and removed at necropsy were placed in buffered neutral 10% formalin. Tissues from animals in the 0, 125, and 1000 ppm groups were processed, sectioned at 5 microns, and stained by the hematoxylin and eosin method for histologic evaluation according to Battelle's Manual for Standard Operating Procedures in Histology and Pathology. The groups fed 250 and 500 ppm rotenone were excluded from initial histopathological examination since these data would be superfluous if either one of two situations existed: (1) chemical-related tumors were found in the 125 ppm low dose group or (2) no chemical-related tumors were found in either the 125 ppm low dose or the 1000 ppm high dose groups. In the first situation, the tumor-inducing threshold dose would be below 125 ppm, and data from the 250 and 500 ppm groups would not add to the question of the carcinogenic nature of rotenone. In the second circumstance, lack of tumors in the high and low dose groups would suggest that the tumor-inducing threshold for rotenone is above 1000 ppm, and that formation of tumors by the intermediate dose would be extremely unlikely. If tumors were found in the 1000 ppm rotenone group, but none in the 125 ppm group, the preserved tissues could have been processed and examined for the establishment of a dietary threshold.

Tissues Removed at Necropsy

Skin (Thoracic)	Kidney	Stomach	Adrenal
Skin (Abdominal)	Ureter	Duodenum	Rib
Mammary Gland	Bladder	Jejunum	Femur
Trachea	Ovary	Ileum	Muscle
Lung	Uterus	Cecum	Cerebrum
Heart	Testicle	Colon	Cerebellum
Abdominal Aorta	Epididymis	Rectum	Medulla
Bronchial Lymph Node	Prostate	Pancreas	Pituitary
Mandibular Lymph Node	Seminal Vesicle	Liver	Spinal Cord
Mesenteric Lymph Node	Salivary Gland	Gall Bladder	Sciatic Nerve
Spleen	Tongue	Thyroid	Eyes
Thymus	Esophagus	Parathyroid	

Selected sections of kidney, spleen, liver, adrenal, and thyroid glands were stained by the Bennhold Congo red method for amyloid detection. Other selected samples of adrenal glands were stained by the Gomori chromaffin staining technique for chromaffin granule detection.

Substantial spontaneous death losses were encountered in all groups of hamsters from this study during the first 12 months of exposure. The deaths were apparently not related to rotenone administration since the losses were as high or higher in controls than in the treatment groups. Most deaths during the first year of the study were associated with a syndrome of cecal hemorrhage and dilatation which often involved the ileum and colon, and in a few instances, the upper small intestine. The syndrome was characterized clinically by sudden death in apparently healthy animals with no prodromal clinical signs. Intestinal and cecal material from affected animals, when passed through a 450 nm filter and injected into the peritoneal cavity of young mice, caused death within 4 to 8 hours in dilutions up to 1:256. Similar filtrates from non-affected hamsters did not produce death in mice. Clostridium perfringens B, C, D, and E antitoxins would not protect against the lethality. The lethal capacity of the filtrates was destroyed by heating to 56° F for 30 minutes. A pathogenic strain of E. coli was isolated from several animals which died of this syndrome. This evidence indicates that a heat-labile

enterotoxin of E. coli may have induced this syndrome. However, unequivocal evidence of this was not obtained since experimental production of the syndrome with the suspected isolates or toxins produced by them was not attempted in other hamsters. The identification of the enterotoxin was not pursued at the request of the EPA Project Officer.

Spontaneous deaths from this syndrome decreased with age and finally subsided after the animals reached approximately one year of age. Spontaneous deaths after one year began to increase again and were primarily a result of systemic amyloidosis which is common in Syrian hamsters.

Due to the relatively large number of early spontaneous deaths, it was mutually agreed by the Project Officer from the Environmental Protection Agency and Battelle's Principal Investigator that microscopic evaluations were performed on animals that died or were terminated after approximately one year on study. Gross pathological examinations were performed on all animals for tumor formation. Tumors were diagnosed at necropsy in 3 animals from either the 500 or 250 ppm dosage groups. Data from microscopic evaluations of these animals are discussed later in this report but are not included in the tabulated data.

Observations

The mean values for hamster body weights are presented in Figure 3. Both 500 and 1000 ppm groups exhibited depressed body weights relative to other test groups, particularly males fed the high dose. This trend may have resulted in part from the decreased feed consumption for these groups during the first six months of the study as seen in Figure 4.

Group mean rotenone consumption values are presented in Figure 5 expressed as milligrams of rotenone consumed per kilogram of body weight per week. Data are plotted for alternate weeks.

Survival data for the hamsters on study are presented in Table 1. Spontaneous deaths were not dose-related for rotenone, nor were there sex-related differences for mortalities in the test groups. Female control animals experienced a high death rate during the final 5 months of the study which may have been related to the enteric infections which were prevalent in this group early in the study.

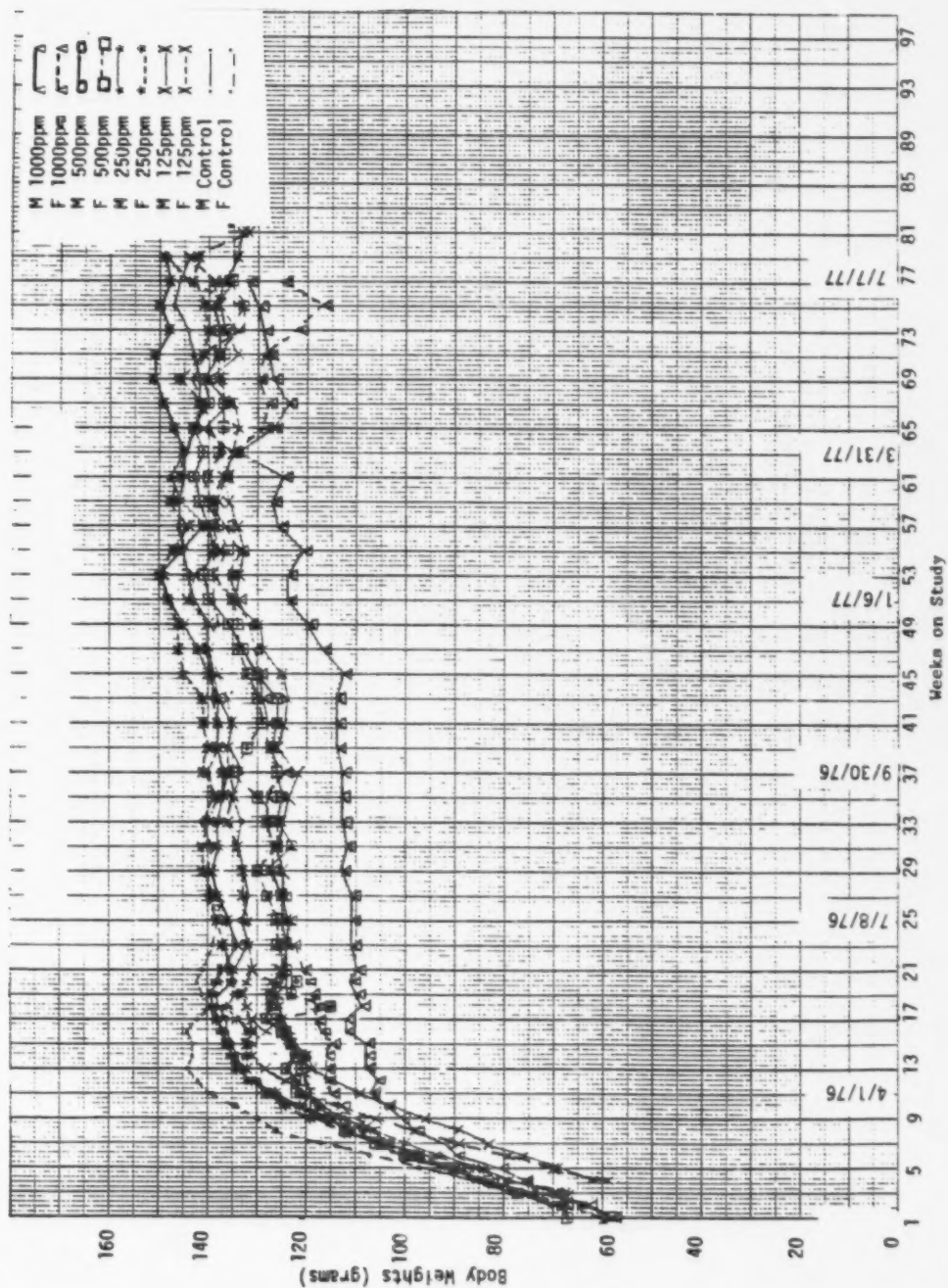


FIGURE 3. Group Mean Body Weights for Hamsters in Carcinogenesis Study

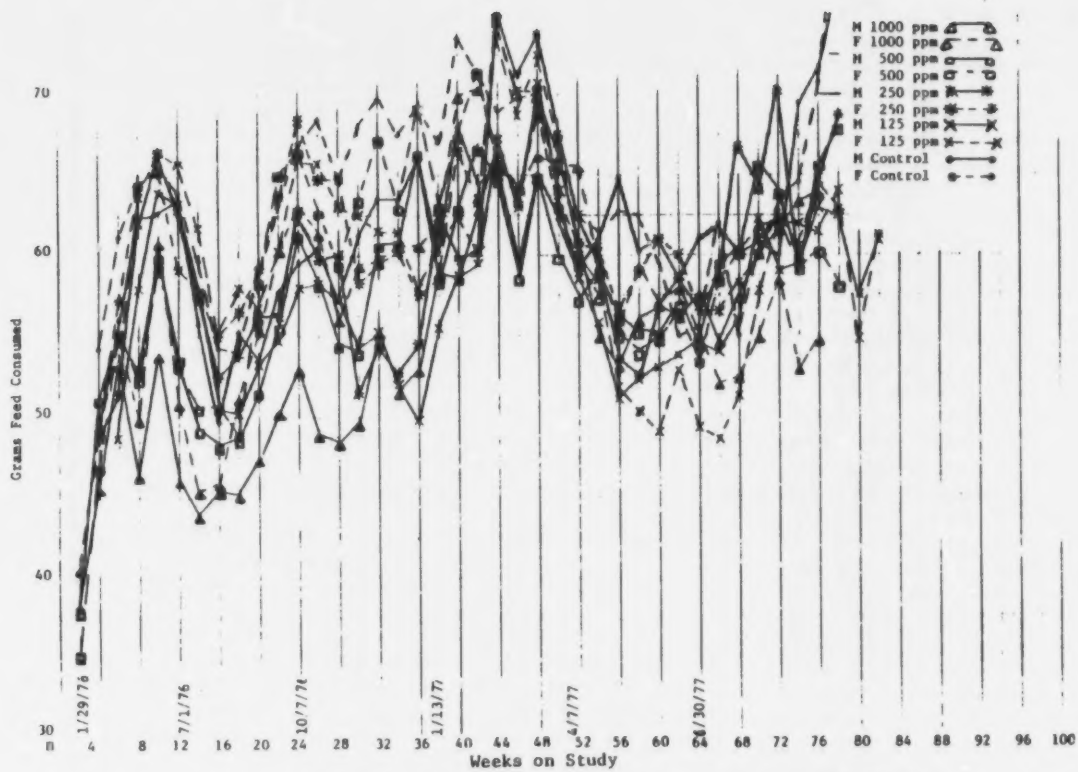


FIGURE 4. Group Mean Rotenone Diet Consumption in Carcinogenesis Study

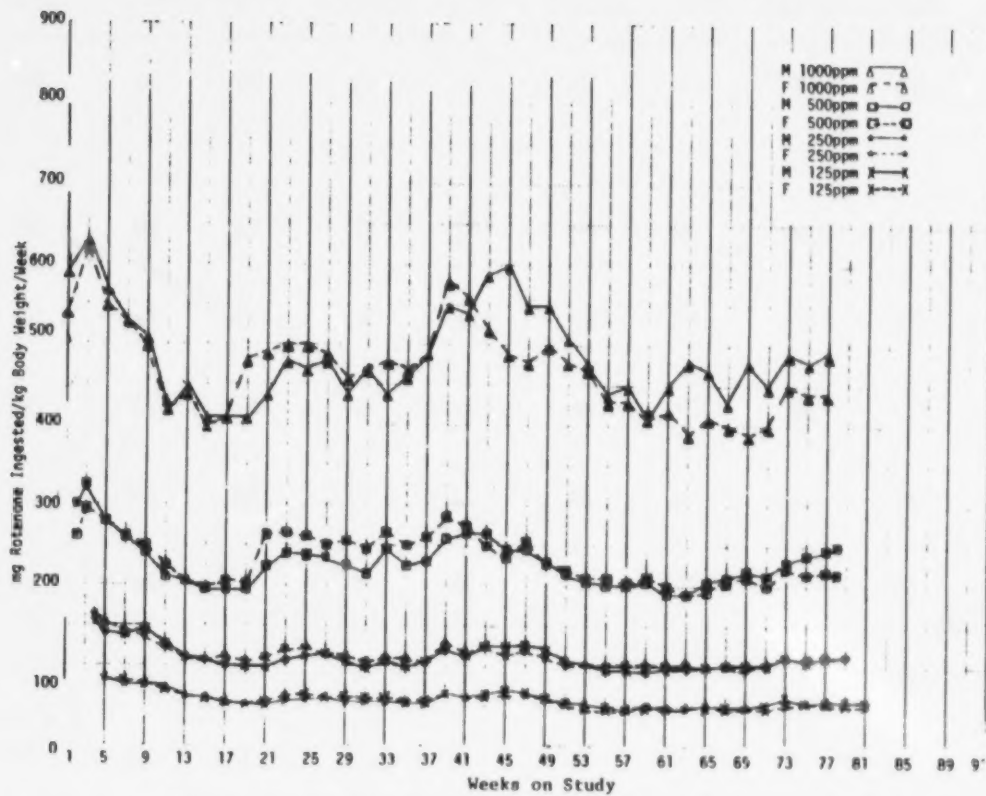


FIGURE 5. Group Mean Rotenone Consumption During Carcinogenesis Study

TABLE 1

HAMSTERS SURVIVING WHILE MAINTAINED ON ROTENONE DIETS

<u>Date</u>	<u>1000 ppm</u>		<u>500 ppm</u>		<u>250 ppm</u>		<u>125 ppm</u>		<u>0 ppm</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
Initial	50	50	50	50	50	50	50	50	50	50
January 1977 (12 months)	33	34	35	30	33	37	35	28	30	28
February	32	33	34	28	31	34	32	26	30	27
March	29	30	33	26	27	30	27	25	30	23
April	27	28	32	26	27	30	26	24	29	20
May	26	27	31	23	25	28	25	22	28	14
June	25	24	29	21	24	27	24	19	25	11
July	25	19	26	20	20	24	24	14	22	6
August (until necropsy)	24	19	25	18	19	20	21	9	22	2

Gross visible lesions encountered at necropsy of hamsters dying spontaneously and in euthanized animals at study termination are presented in Tables 2 and 3 respectively. Notable lesions encountered in animals dying spontaneously were nephrosis due to apparent amyloid deposition, centrilobular congestion and necrosis of the liver, vegetative thrombosis generally in the left atrium of the heart, pulmonary congestion, hemorrhage and pneumonia, as well as atrophy or hypoplasia of the testicles.

Several tumors were also apparent at necropsy. Ovarian tumors were noted in female animals number 94 of the 1000 ppm group, and number 285 of the 0 ppm group; masses involving leg muscles were found in animal number 69 (female) of the 1000 ppm group, and animal number 291 (female) of the 0 ppm group. A renal tumor was suspected in animal number 128 (male) of the 500 ppm group, and a cystic adenoma was found in animal 472 (female) of the 125 ppm group; possible thyroid tumors were found in female hamsters 451 of the 125 ppm group, and 284 and 286 of the 0 ppm group. An adrenal tumor was suspected in animal number 61 (female) of the 1000 ppm group; and a lymphoid neoplasm was present in several tissues from animal number 383 (female) of the 250 ppm group.

Gross lesions observed in hamsters euthanized at the termination of the study are described in Table 3. Euthanized hamsters had fewer and less severe inflammatory intestinal and pulmonary lesions than animals that died spontaneously. The incidence of amyloid-induced nephrosis in hamsters terminated at the end of the study was higher, and was more severe than that present in hamsters with spontaneous deaths. The incidence of hepatic or biliary cysts in the liver also increased with age. Grossly visible tumors or focal hyperplasias of the adrenal glands were noted in male hamsters 4 and 21 of the 1000 ppm group, number 107 of the 500 ppm group, number 402 and 425 of the 125 ppm group, and number 220 of the 0 ppm group. A chondroma of the costocondral junction of the rib was found in animal number 83 (female) of the 1000 ppm group.

Results of Histopathological evaluations of animals from the 1000, 125 and 0 ppm dosage groups which died or were euthanized are summarized in Table 4.

TABLE 2. GROSS LESIONS IN HAMSTERS THAT DIED SPONTANEOUSLY
(INCIDENCE AND PERCENT AFFECTED)

	Dosage Level and Number in Group									
	1000 ppm		500 ppm		250 ppm		125 ppm		0 ppm	
	57	%	57	%	61	%	70	%	76	%
Enteritis	26	45.6	23	40.3	20	32.7	37	52.8	22	28.9
Typhilitis	27	47.3	33	57.8	24	39.3	28	40.0	33	43.4
Colitis	8	14.0	9	15.7	8	13.1	8	11.4	6	7.8
Nephrosis	10	17.5	8	14.0	17	27.8	25	35.7	28	36.8
Liver Centrilobular Congestion and Necrosis	9	15.7	12	21.0	18	29.5	13	18.5	17	22.3
Pulmonary Congestion and Hemorrhage	8	14.0	20	35.0	19	31.1	20	28.5	29	38.1
Pneumonia	10	17.5	4	7.0	8	13.1	1	1.4	4	5.2
Heart Arterial or Ventricular Thrombosis	4	7.0			4	6.5	5	7.1	7	9.2
Liver Hepatic or Biliary Cysts	2	3.5	2	3.5	3	4.9	5	7.1	10	13.1
Gastric Ulceration	2	3.5	1	1.7	6	9.8	5	7.1	1	1.3
Rectal Prolapse and Intussusception					1	1.6	1	1.4	2	2.6
Testicular Atrophy or Hypoplasia	6	10.5	1	1.7	4	6.5	9	12.8	1	1.3
Ovarian Tumor or Cyst	1	1.7			1 cyst	1.6	1 cyst	1.4	1	1.3
Tumor (Muscle of Leg)	1	1.7							1	1.3
Renal Tumor			1	1.7			1	1.4		
Spleen Focal Hyperplasia or Tumor Mass	1	1.7			1	1.6			2	2.6
Thyroid Focal Hyperplasia or Tumor Mass							1	1.4	2	2.6
Adrenal Focal Hyperplasia or Tumor Mass	1	1.7								
Salivary Gland Focal Hyperplasia or Tumor Mass							1	1.4		
Tumor, Lymphosarcoma					1	1.6				

TABLE 3. GROSS LESIONS IN HAMSTERS TERMINATED AT THE END OF THE STUDY
(INCIDENCE AND PERCENT AFFECTED)

	Dosage Level and Number in Group									
	1000 ppm		500 ppm		250 ppm		125 ppm		0 ppm	
	43	%	43	%	39	%	30	%	24	%
Enteritis					4	10.2	5	16.6	3	12.5
Typhilitis	1	2.3					2	6.6		
Colitis					1	2.5				
Nephrosis	9	20.9	21	48.8	27	69.2	19	63.3	16	66.6
Liver Centrilobular Congestion and Necrosis	1	2.3	5	11.6	8	20.5	5	16.6	3	12.5
Pulmonary Congestion and Hemorrhage	6	13.9	3	6.9	12	30.7	1	3.3	9	37.5
Heart Atrial or Ventricular Thrombosis			4	9.3	2	5.0				
Liver Hepatic or Biliary Cysts	10	23.2	5	11.6	10	25.6	3	9.9	5	20.8
Gastric Ulceration	1	2.3								
Testicular Atrophy or Hypoplasia	1	2.3	1	2.3			1	3.3	2	8.3
Ovarian Cysts					2	5.0	1	3.3		
Eye Microphthalmic Appearance	1	2.3			1	2.5				
Spleen Focal Hyperplasia or Tumor	2	4.6			5	12.8	3	9.9		
Thyroid Congestion	3	6.9								
Adrenal Congestion	2	2.4								
Adrenal Focal Hyperplasia or Tumor	3	6.9	1	2.3			3	9.9	1	4.1
Rib Tumor Chondroma	1	2.3								
Epididymus Sperm Granuloma									1	4.1
Skin Focal Hyperplasia of Dermal									1	4.1

TABLE 4. DISTRIBUTION OF HAMSTERS
EVALUATED MICROSCOPICALLY

	1000 ppm		125 ppm		0 ppm	
	Male	Female	Male	Female	Male	Female
Spontaneous Deaths	8	14	11	17	8	25
Euthanized	24	19	21	9	22	2
Total	32	33	32	26	30	27

The predominant non-neoplastic microscopic alterations encountered in all groups reviewed were associated with changes due to age related amyloid deposition in the kidneys, liver, spleen, and adrenals (Table 5). The pattern of change found in the kidneys was retraction of the capsular surface with associated tubular degeneration and regeneration with the deposition of an amyloid material in the tubular interstitial tissues, generally of the cortex. The mesangium of the glomerular tuft was often thickened and contained a similar lightly-stained eosinophilic material; Bowman's capsule was also thickened in some instances. The general picture was that of atrophied misshapened nephron units with dilated collecting tubules filled with brightly staining eosinophilic protein material. The normal liver architecture was altered by rather thick deposits of light pink amyloid-related material in and between the hepatocytes and vascular tissues of the portal triads that often become confluent between adjacent triads. The splenic red pulp was often completely filled with the pale eosinophilic material with only small lymphoid nodules disturbing the monotonous pink fields. The adrenal cortex was also a deposition site for the lightly staining amyloid-like material, where it was found in or between cortical cells. Female hamsters from all three dosage groups had a higher incidence of amyloid-like material deposited between follicles in the thyroid than did males. Thrombosis of the left atrium was present in all female groups but was not found in any male reviewed microscopically. Pulmonary lesions ranged from mild alveolar septal congestion to bronchiolar pneumonia of a moderate severity. Varying degrees of enteritis, typhlitis, and colitis were observed in all groups. Testicular atrophy was present to some extent in all three groups of male animals. Numerous other lesions of lower incidence, less significance, or random occurrence are listed in Table 5, and will not be discussed further.

Tables 6 and 7 list the tumors and various types of hyperplasias which occurred in the different dosage groups. Adrenal cortical carcinomas were observed in the group given 1000 ppm (2 in females, 1 in male). Adenomas and hyperplasias of the adrenal cortex were common in all dosage groups, and were the most consistent lesions noted in the male and female groups examined. Three animals from the medium dosage groups had tumors

TABLE 5. MICROSCOPIC LESIONS BY DOSAGE GROUP AND SEX
(EXCLUDING NEOPLASMIC AND HYPERPLASTIC LESIONS)

Diagnoses	0 ppm				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected	% Number in Group	No. Affected	% Number in Group	No. Affected	% Number in Group	No. Affected	% Number in Group	No. Affected	% Number in Group	No. Affected	% Number in Group
Kidney Amyloidosis	25	(92.5)	26	(86.6)	21	(80.7)	15	(44.8)	28	(84.8)	8	(25.0)
Kidney Tubular Degeneration and Regeneration	21	(77.7)	24	(80.0)	25	(96.1)	30	(93.7)	25	(75.7)	23	(71.8)
Kidney Glomerular Congestion	21	(77.7)	8	(26.6)	23	(88.4)	2	(6.2)	20	(60.6)	2	(6.2)
Liver Amyloidosis	2	(7.4)	3	(10.0)	9	(34.6)	4	(12.5)	17	(54.5)	7	(21.8)
Liver Hepatocyte Vacuolation	11	(40.7)	3	(10.0)	7	(26.9)	3	(9.3)	12	(36.3)	3	(9.3)
Liver Bile Duct Hyperplasia	11	(40.7)	14	(46.6)	12	(46.1)	18	(55.0)	22	(66.6)	6	(18.7)
Liver Sinusoidal Congestion	5	(18.5)	3	(10.0)	15	(57.6)	3	(9.3)	20	(60.6)	4	(12.5)
Liver Bile Duct Hyperplasia	2	(7.4)	13	(43.3)	21	(80.7)	8	(25.0)	72	(66.6)	5	(15.6)
Liver Centrilobular Degeneration and Necrosis	3	(11.1)	6	(20.0)	2	(7.6)	14	(43.7)			5	(15.6)
Liver Extramedullary Hematopoiesis												
Liver Hepatic Suppurative Necrosis	14	(51.8)	1	(3.3)			1	(3.1)			1	(3.1)
Spleen Amyloidosis			3	(10.0)	21	(80.7)	3	(9.3)	18	(54.5)		
Spleen Lymphoid Depletion			1	(3.3)	5	(19.2)	2	(6.2)	2	(6.0)		
Spleen Lymphocyte Depletion			9	(30.0)	13	(50.0)	7	(21.8)	10	(30.3)	2	(6.2)
Lung Bronchiolar Epithelial Hyperplasia	1	(3.7)	3	(10.0)	4	(15.3)	5	(15.6)	4	(12.1)	2	(6.2)
Lung Pulmonary Edema	10	(37.0)	3	(10.0)	5	(19.2)	2	(6.2)	3	(9.0)		
Lung Pulmonary Congestion	13	(48.1)	13	(43.3)	5	(19.2)	9	(28.1)	13	(39.3)	8	(25.0)

TABLE 5. (Continued)

Diagnoses	0 ppm				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected	%	No. Affected	%	No. Affected	%	No. Affected	%	No. Affected	%	No. Affected	%
	27		30		76		32		33		32	
Long Bronchiolar Pneumonia	7	(25.9)	1	(3.3)	5	(19.2)	2	(6.2)	4	(12.1)		
Long Interstitial Pneumonitis	2	(7.4)			4	(15.3)			7	(21.2)		
Long Peribronchiolar fibrosis focal											1	(3.1)
Heart Atrial Thrombosis	14	(51.8)			5	(19.2)			2	(6.0)		
Heart Ventricular Thrombosis	4	(14.8)	1	(3.3)	2	(7.6)						
Heart Myocardial Degeneration, Mild	5	(18.5)	5	(16.6)	3	(11.5)	2	(6.2)	5	(15.1)	2	(6.2)
Adrenal Cortex Amyloidosis	12	(44.4)	4	(13.3)	17	(65.3)	4	(12.5)	18	(54.5)	3	(9.3)
Adrenal Medullary Cell Degeneration	6	(22.2)	9	(30.0)	3	(11.5)	4	(12.5)	7	(21.2)	5	(15.6)
Eye Lenticular Degeneration Vacuolar	1	(3.7)	2	(6.6)	1	(3.8)	4	(12.5)	3	(9.0)	2	(6.2)
Intestine Lamina Propria Amyloidosis	5	(18.5)			3	(11.5)	1	(3.1)	2	(6.0)		
Enteritis	1	(3.7)	8	(26.6)	14	(53.8)	11	(34.3)	12	(36.3)	8	(25.0)
Typhilitis	4	(14.8)	14	(46.6)	9	(34.6)	13	(40.6)	14	(42.4)	8	(25.0)
Gastritis	1	(3.7)	7	(23.3)	3	(11.5)	4	(12.5)	5	(15.1)	2	(6.2)
Thyroid Amyloidosis	8	(29.6)	2	(6.6)	11	(42.3)	1	(3.1)	10	(30.3)	1	(3.1)
Heart Diastrophic Mineralization	3	(11.1)					1	(3.1)	2	(6.0)		
Lymph Node Mandibular Lymphadenitis	9	(33.3)			8	(30.7)	2	(7.6)	3	(9.0)	1	(3.1)
Lymph Node Mesenteric Lymphadenitis	5	(18.5)	6	(20.0)	10	(38.4)	6	(18.7)	5	(15.1)	4	(12.5)
Heart Atrial-Ventricular Valve Thickening	7	(25.9)	6	(20.0)	9	(34.6)	1	(3.1)	6	(18.1)	3	(9.3)

TABLE 5. (Continued)

Diagnoses	0 ppm				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group
Heart Atrioventricular Valve Thrombosis												
Ovary Abscess	1	(3.7)							1	(3.0)	1	(3.1)
Brain Cerebrum Perivascular Cuff Mild	3	(11.1)										
Gonadsitis NOS												
Kidney, Adrenal Cortex Subcapsular Rest			1	(3.3)								
Seminal Vesicle, Mucosa Papillary Hyperplasia			1	(3.3)								
Eye, Microphthalmia with Distortion									1	(3.0)		
Passive Congestion Generalized									1	(3.0)		
Ecopic Adrenal Rest, Brown Fat									1	(3.0)		
Skeletal Muscle, Necrosis									1	(3.0)		
Mammary Gland, Acinar Cell Hyperplasia Focal									1	(3.0)		
Tongue Glossitis											1	(3.1)
Adrenal Cortico-Medullary Cyst	1	(3.7)	1	(3.3)					1	(3.0)		
Ovary, Cystic Follicle												
Fallopian Tube Cyst					2	(7.6)						
Liver, Bile Duct Ectasia					2	(7.6)						
(Cholangiomatosis)					3	(11.5)						

TABLE 5. (Continued)

Diagnoses	0 ppm				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group	No. Affected	No. in Group
Pituitary Pars Distalis Vacuolar Degeneration	7	(25.9)	13	(43.3)	4	(15.3)	9	(28.1)	8	(24.2)	10	(31.2)
Parathyroid Cysts Multilocular	1	(3.7)										
Cerebrum Ischemic Necrosis	1	(3.7)										
Heart Atrium Bone Marrow			1	(3.3)								
Bone Marrow Rib Cellular Arrest			1	(3.3)	1	(3.8)			1	(3.0)		
Salivary Gland Acinar Cell Atrophy	1	(3.7)										
Salivary Gland Adenitis	1	(3.7)										
Ovary Cytoplasmic Vacuolar Alteration	3	(11.1)										
Lymph Node Mandibular Granuloma			1	(3.3)								
Epididymis Sperm Granuloma			1	(3.3)								
Seminal Vesicles Mineralization			1	(3.3)								
Brain Choroid Plexus Dystrophic Mineralization			1	(3.3)								
Ileum Ileitis Proliferative			2	(6.6)								
Stomach Fundic Mucosa Mineralization			1	(3.3)			1	(3.8)				
Thyroid Follicular Cysts Multilocular					3	(11.5)	2	(7.6)	4	(12.1)	1	(3.1)
Stomach Gastritis					2	(7.6)			1	(3.0)		
Testicle Atrophy and Seminiferous Cell Degeneration			4	(13.3)			8	(25.0)			2	(6.2)
Stomach Gastric Ulceration									1	(3.0)	1	(3.1)

TABLE 5. (Continued)

Diagnoses	0 ppm			175 ppm			1000 ppm		
	Female		Male	Female		Male	Female		Male
	No. Affected	%	No. in Group	No. Affected	%	No. in Group	No. Affected	%	No. in Group
Stomach, Cardia, Papillary Fronds			30			32			32
Pancreas Acinar Cell Atrophy				2	(7.6)				
Cecum Proliferative Cystic Typhilitis				2	(7.6)	3	(9.3)		
Pancreas Branchial Arch Cysts				1	(3.8)				
Nose, Inflammation				1	(3.8)				
Tongue, Dystrophic Mineralization						2	(6.2)		
Heart, Coronary Artery Mineralization						1	(3.1)		
Heart, Coronary Artery Sclerosis						3	(9.3)		
Testicle, Necrotizing Vasculitis Focal						1	(3.1)		

TABLE 6. NEOPLASTIC AND HYPERPLASTIC LESIONS

	0 ppm				125 ppm				1000 ppm			
	Female		Male		Female		Male		Female		Male	
	No.	Affected	No.	Affected	No.	Affected	No.	Affected	No.	Affected	No.	Affected
	27		30		26		32		33		32	
Leg Muscle Fibrosarcoma	1	(3.7)										
Leg Muscle Undifferentiated Sarcoma Metastatic												
Ovary Granulosa Cell Tumor	1	(3.7)			1	(3.8)			1	(3.0)		
Thyroid Parafoollicular Cell Adenoma	1	(3.7)	2	(6.6)					2	(6.0)		
Rib Chondroma									1	(3.0)		
Pituitary Adenoma	2	(7.4)							1	(3.0)		
Spleen Hemangioma												
Thyroid Follicular Cell Carcinoma							1	(3.1)			1	(3.1)
Trachea Mucosal Carcinoma											2	(3.1)
Adrenal Medulla Pheochromocytoma											1	(3.1)
Pancreas Adenoma	1	(3.7)										
Adrenal Cortical Cell Hyperplasia	13	(48.1)	11	(36.6)			15	(46.8)	15	(45.4)	19	(59.3)
Adrenal Cortex Adenoma	5	(18.5)	8	(26.6)	7	(26.9)	8	(25.0)	6	(18.1)	8	(25.0)
Adrenal Cortex Carcinoma									2	(6.0)	1	(3.1)
Spleen Berculoendothelial Cell Sarcoma							1	(3.1)				
Thyroid Parafoollicular Cell Hyperplasia			2	(6.6)								
Skin Sebaceous Gland Hyperplasia			1	(3.3)								
Adrenal Medulla Hyperplasia												
Thyroid Follicular Cell Adenoma Cystic							1	(3.1)				
Kidney Papillary Cystic Adenoma Cortex							1	(3.8)				

TABLE 7. MICROSCOPIC DIAGNOSES OF NEOPLASIA BY ANIMAL NUMBER *

Lesion	0 ppm			125 ppm			1000 ppm		
Adrenal Cortex Adenoma	<u>207</u>	<u>241</u>	285	<u>402</u>	<u>431</u>	476	<u>5</u>	<u>39</u>	99
	<u>211</u>	<u>245</u>	286	<u>408</u>	<u>433</u>	477	<u>13</u>	<u>41</u>	100
	<u>217</u>	<u>250</u>		<u>411</u>	<u>457</u>	496	<u>15</u>	<u>61</u>	
	<u>226</u>	257		<u>413</u>	461		<u>21</u>	66	
	<u>228</u>	277		<u>414</u>	470		<u>28</u>	90	
	<u>229</u>			<u>427</u>	472		<u>34</u>	94	
Adrenal Cortex Carcinoma							<u>4</u>	73	
							60		
Adrenal Medulla Pheochromocytoma							<u>12</u>		
Trachea Mucosa Carcinoma							<u>20</u>		
Ovary Granulosa Cell	285			497			94		
							96		
Spleen Hemangioma							<u>44</u>		
Kidney Papillary Cyst Adenoma				472					
Rib Chondroma							83		
Thyroid Cystic Follicular Adenoma				451					
Thyroid Follicular Carcinoma				<u>415</u>			<u>30</u>		
Thyroid Parafoallicular Cell Adenoma	284	<u>242</u>	<u>245</u>						
Parathyroid Adenoma	286			<u>415</u>					
Spleen Reticulum Cell Sarcoma				<u>447</u>					
Pituitary Pars Distalis Adenoma	279	286					68		
Fibrosarcoma Leg	291								
Undifferentiated Sarcoma NOS							69		

* Males are underlined.

which were evident at necropsy. Microscopically these were diagnosed as renal nephroblastoma for male hamster number 128 (500 ppm), lymphosarcoma in number 383 (female, 250 ppm), and splenic hemangioma in number 386 (female, 250 ppm).

Acinar cell hyperplasia occurred in the mammary tissue of one female from the 1000 ppm group. There were no neoplastic or other lesions present in mammary tissue from hamsters in this study. Adrenal cortical adenomas and hyperplasias occurred with similar frequency in all groups. However, 3 adrenal cortical carcinomas occurred in the group that received 1000 ppm rotenone in their diet; one male (number 4) and one female (number 73) went to study completion, while another female (number 60) died June 1, 1977, two months prior to study termination. Analysis of these data by a Chi-square 2 X 3 contingency scheme indicates that the appearance of this tumor in the high dose group (compared to the non-occurrence in the 0 and 125 ppm groups) is significant between the 90 and 95 percent confidence levels. However, because the control group represented a younger population of hamsters due to a larger number of early deaths, this comparison is not valid since these tumors are known to develop spontaneously in aging hamsters (3). The significance of these carcinomas in the highest dosage group and their absence from other groups is not clear. Although a correlation between these adrenal cortical carcinomas and rotenone treatment cannot be ruled out, evidence for a cause-effect relationship is inconclusive.

The lesions encountered in this 18-month study are similar in nature to those reported by Pour et al (1-4), Chesterman (5), and Gleiser et. al. (6) in large hamster colonies retained for life time studies. Aging hamsters are predisposed to development of renal, splenic, hepatic, and adrenal lesions related to amyloid deposition. In this study, no substantial histopathological differences were seen between treatment groups. Males in this study had less amyloid depositions in the liver, spleen, adrenals, and thyroid than females. No profound differences in the incidence of non-neoplastic pathologic lesions were observed between rotenone-treated animals at various dose levels and the control group. No pathologic alterations of note were seen in any dose group relative to mammary gland changes.

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